临床血流感染沙门菌的分型及同源性与耐药性分析*

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摘 要：目的 探讨临床血液感染沙门菌血清分型、基因同源性及耐药质粒和细菌耐药性的关系。**方法** 收集武汉大学中南医院2015～2017年临床血流感染分离的10株沙门菌。采用法国梅里埃Vitek 2 Compact全自动鉴定药敏检测系统进行鉴定和药敏实验，按国家标准对沙门菌进行血清学分型，用肠杆菌科基因间重复序列的聚合酶链反应（ERIC-PCR）对其进行基因分型，采用Cluster 3.0软件对PCR扩增产物进行聚类分析。**结果** 10株沙门菌分为3种血清群，A群1株，B群3株，D群6株，D群是优势血清群，约占60.0%；10株沙门菌中2，3，4，5和8号菌株含有质粒，不含质粒的是1，6，7，9和10号菌株；10株沙门菌对氨苄西林耐药率为80.0%，左氧氟沙星中介率80.0%，对三、四代头孢及碳青霉烯类100.0%敏感，对复方磺胺类敏感率为80.0%；4号和10号菌株、8号和9号菌株的同源性超过80.0%，1号和2号，5号和6号菌株的同源性大于70.0%。**结论** 沙门菌存在耐药质粒，质粒的多少表明耐药程度的高低；沙门菌对氨苄西林及氨苄西林/舒巴坦耐药率较高，对头孢他啶敏感率高，因此治疗血流感染时可选用三、四代头孢或亚胺培南，采用降阶梯治疗。

关键字：沙门菌；血清分型；基因分型；耐药性；肠杆菌科基因间重复序列的聚合酶链反应；同源性分析
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Typing and Homology and Resistance Analysis of Salmonella in Clinical Bloodstream Infection

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Abstract: Objective To investigate the relationship between serotyping, gene homology, drug-resistant plasmids and bacterial resistance of *Salmonella* in clinical blood infection. **Methods** 10 strains of *Salmonella* isolated from clinical blood stream infection in Zhongnan Hospital of Wuhan University during the past 2015～2017 years were collected. The strains identification and drug sensitive test were performed by French BioMerieux Vitek 2 Compact automatic identification and drug sensitivity detection system, serological typing was carried out according to the national standard, genotyping was conducted by enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), clustering analysis was done using Cluster 3 software for PCR amplification products. **Results** 10 strains of *Salmonella* contained 3 serum groups, 1 strain of A group, 3 strains of B group, 6 strains of group D respectively, D group was the dominant serogroup, accounted for 60.0%, 10 strains of *Salmonella* containing plasmid were strain 2, strain 3, strain 4, strain 5, strain 8, and no plasmid containing bacteria strain 1, strain 6, strain 7, strain 9, strain 10, 10 strains *Salmonella* resistant rate to ampicillin were 80.0%, levofloxacin intermediary rate reached 80%, but their sensitive rates to the third or fourth generation cephalosporins and carbapenems were 100.0%, their sensitive rate to compound sulfanilamide was 80.0%. The gene homology between strain 4 and strain 10 was more than 80.0%, strain 9 and strain 8 homology was also over 80.0%, the gene homology between strain 1 and strain 2 and between strain 5 and strain 6 was over 70.0%. **Conclusion** There were some plasmids on some *Salmonella* strains which implied the level of drug resistance. *Salmonella* resistance rates to ampicillin and ampicillin/sulbactam were high, but they were very sensitive to ceftazidime, and so the treatment of bloodstream infection has better to select the third or fourth generation ceftazidime or imipenem and employ deescalation therapy strategy.

Keywords: *Salmonella*; serotyping; genotyping; drug resistance; ERIC-PCR; homology analysis

沙门菌是引发食源性疾病的主要病原菌之一[1]，沙门菌感染主要通过被沙门菌污染的肉类、蛋类、乳制品等而引起发病，沙门菌经口传播，早期进入血流，感染后多表现为败血症。沙门菌的耐药问题日益严重，以前广谱的头孢菌素和氟喹诺酮类抗生素推荐用于治疗非伤寒沙门菌感染[2]，但头孢曲松和环丙沙星耐药的沙门菌不断被报道[3]。本文对临床血流感染沙门菌分型及同源性和耐药性关系的分析，为临床血流感染的检测和指导临床用药提供参考依据。

1 材料与方法

1.1 菌株来源 10株沙门菌来自于2015～2017年武汉大学中南医院微生物室，分离自本院发热病人的血液中。

1.2 主要试剂 MH琼脂、血平板由广州迪景公司提供，鉴定卡和AST-GN13药敏卡片由旭航公

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司提供,沙门菌抗原诊断血清购自浙江宁波天润,TaqDNA聚合酶和dNTPs由Fermantas公司提供,DNA Marker 100bp由广州东盛提供,质粒少量制备试剂盒购自上海捷瑞生物工程有限公司,ERIC引物由北京擎科新业生物技术有限公司合成。

1.3 实验方法

1.3.1 细菌鉴定及药敏实验:采用Vitek compact 2全自动细菌鉴定及药敏分析仪进行沙门菌的鉴定,药敏卡AST-GN13进行药敏试验,测定最小抑菌浓度(MIC),结果判读参照美国临床实验室标准化协会(CLSI)M100-S23标准,质控菌株为大肠杆菌ATCC25922,购自卫计委临床检验中心。

1.3.2 血清型鉴定:用A～F多价O血清做玻片凝集实验,生理盐水对照,若有凝集,再依次用O单价血清做凝集,根据凝集实验结果判断O群,然后根据O抗原进行H抗原和鞭毛抗原的血清凝集,必要时进行诱导实验,最后根据O抗原和H抗原判断沙门菌的血清型别。

1.3.3 细菌质粒的提取及基因的检测:质粒的提取采用的是质粒小量试剂制备盒提取,按照试剂说明书操作进行。

1.3.4 PCR扩增体系:总体积为10 μl,包含DNA模板2 μl,dNTP(10 mmol/L)0.5 μl,上、下引物各为0.5 μl,10×PCR缓冲液1.2 μl,DNA聚合酶(5 U/μl)0.1 μl,双蒸水5.2 μl。

1.3.5 反应条件:预热95℃3 min变性,96℃30 s,退火42℃50 s,延伸72℃1 min,30个循环,72℃5 min。

1.4 聚类分析 将ERIC-PCR电泳谱带数据转换成矩阵,用"1"和"0"代表谱带的有和无,然后运用Cluster 3.0软件对10株扩增产物进行相似性分析,聚类分法选用非加权组平均法。

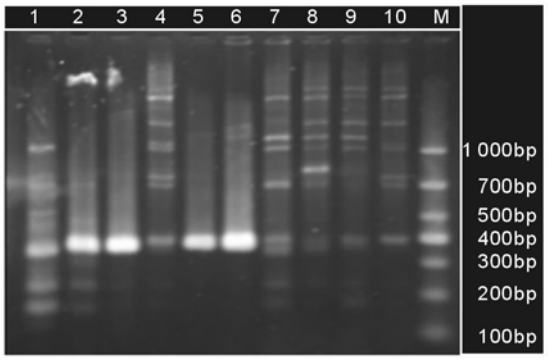
2 结果

2.1 沙门菌血清学分型结果 10株沙门菌均符合三糖铁斜面红色,底部黄色,底部出现部分或全部黑色,鉴定仪鉴定为沙门菌,按照国标要求对沙门菌进行血清学分型,必要时进行诱导和传代,血清型A型1株,为甲型伤寒沙门菌;B型3株,2株乙型副伤寒沙门菌和1株鼠伤寒沙门菌;D型为6株,4株为伤寒沙门菌,2株为肠炎沙门菌。患者年龄19～81岁,男女患者比为9∶1.

2.2 沙门菌质粒的分型 对10株沙门菌质粒进行琼脂糖凝胶电泳,10株沙门菌含有质粒的是2,3,4,5,8号菌株,不含质粒的是1,6,7,9,10号菌株,2号菌株含有2种质粒,分子量约为20 kb和35 kb;8号菌株含有2种质粒,分子量约为15 kb和20 kb;3,4,5号菌株含有分子量相同的1种质粒,分子量约为35 kb。

2.3 10株沙门菌对抗生素的耐药性 依据CLSI2017年指南,我们对4类抗生素进行药敏试验,10株沙门菌中,氨苄西林有2株为敏感,敏感率为20.0%(2/10),头孢他啶敏感率为100.0%(10/10),甲氧苄啶-磺胺甲唑敏感率为80.0%(8/10),左氧氟沙星1株敏感,8株中介,1株耐药,敏感率为10.0%(1/10)。

2.4 沙门菌的基因分型 对10株沙门菌进行ERIC-PCR扩增,结果见图1。每种菌株有不同的条带,最多的有10条带,最少的有2条带。



(M为marker,1～10为菌株号)

图1 10株沙门菌ERIC-PCR扩增产物图谱

2.5 沙门菌的同源性分析 用Cluster 3.0软件对10株沙门菌扩增产物做聚类分析,4号和10号菌株的相似性最高,达85.70%,其次是8号和9号菌株相似性为83.70%,具有高度同源性,7号菌株与这两株菌相似性为72.50%。1号与2号菌株、5号和6号菌株的相似性分别为73.00%,70.70%,具有一定的同源性。3号与1号、2号菌株的相似性仅为44.70%,不具有同源性。结果见图2。

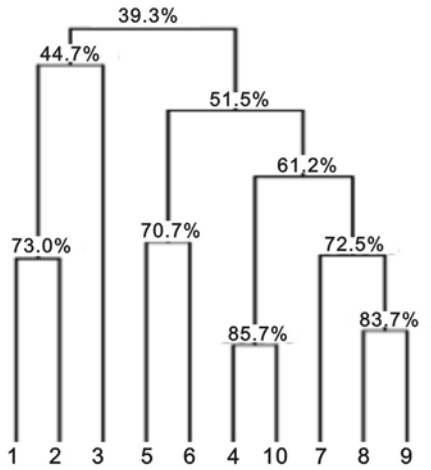


图2 沙门菌聚类分析图谱

3 讨论 抗生素的不合理使用导致沙门菌对青霉素和喹诺酮类药物耐药率越来越高,(下转88页)

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(上接84页)国内外已有文献报道,沙门菌在某些地区已经出现喹诺酮类药物高水平耐药,主要是由于喹诺酮耐药决定区(QRDR)基因的突变[4]和喹诺酮耐药基因qnr及aac(6')-Ib-cr的携带[5],gyrA的点突变被认为在高喹诺酮类耐药革兰阴性菌的所有耐药机制中占主导地位,也被严海忠等[6]人从鼠伤寒沙门菌中得到印证。质粒介导的耐药对细菌耐药起着一定作用,质粒检出的数量越多,多重耐药性越强[7]。本研究的10株沙门菌中,检出3种类型的质粒谱型,都是大分子质粒,如含有2种质粒的2,8号菌株,含有一种质粒的3,4,5号菌株,这些菌株的多重耐药率高于不含质粒的菌株。

10株沙门菌对氨苄西林和左氧氟沙星敏感率较低,对氨苄西林耐药率为80.0%,左氧氟沙星耐药率为10%,但左氧氟沙星中介率高达80.0%,因此青霉素类和喹诺酮类已不适合一线抗生素治疗,沙门菌对第三代头孢菌素及磺胺类药物仍有较高的敏感率,可作为沙门菌感染患者的经验性用药,对沙门菌引起的血流感染也可采用亚胺培南进行降阶梯治疗。

临床医生应重视沙门菌血流感染的病原学诊断,合理使用抗生素,尽量避免选用氨苄西林及左氧氟沙星药物,可选择第三代头孢及碳青霉烯类抗生素来治疗。

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